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Dental wash: a problematic method for extracting microfossils from teeth[☆]

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Abstract

A variety of microfossils, originating from plant foods, become trapped in the dental calculus matrix. Processing of dental calculus allows extraction of these microfossils. The resulting data can be used to reconstruct diet at the individual and population levels as the identification of microfossils like starch grains and phytoliths to the generic level, and sometimes to the species level, is possible. However, in some archaeological sites, dental calculus deposits do not preserve well enough to be processed. To prevent the loss of information in such cases, we present a technique, called “dental wash”. It permits extracting microfossils from cryptic dental calculus deposits. In the two experimental archaeological cases presented herein we identified phytoliths, starch grains as well as a diatom fragment with this method, whereas in a control sample no microfossils were found. Moderate damage to the teeth was detected when they were already friable due to poor preservation. Minor damage to the surface of well-preserved teeth was observed. This indicates that the proposed method is efficient in recovering microfossils, but unacceptable because of damage to teeth. If the method can be refined, it will expand the potential of dental calculus analysis to a greater range of archaeological sites.

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1. Introduction

The analysis of vegetal microscopic residues has been useful in the reconstruction of daily life of past populations. This kind of analysis has shed light on crucial issues for archaeology, such as stone tool function and artifact production, and dietary change associated with the advent of agriculture (Danielson and Reinhard, 1998; Piperno and Holst, 1998; Piperno et al., 2000; Shafer and Holloway, 1979; Zarrillo and Kooyman, 2006). An innovative approach to the study of ancient diet is the analysis of dental calculus (Fig. 1) (Middleton and Rovner, 1994; Fox et al., 1996; Nelson, 1997; Reinhard et al., 2001a,b).

In these studies the identification of microfossils could render information about specific plants eaten and manipulated with the teeth (Cummings, 1992; Pearsall, 1989; Piperno, 1989). It is an ideal complementary method to stable isotopic reconstruction, because the dental calculus provides information about foods eaten a short time before death (from days to weeks, depending on the size of the deposit), whereas isotopic analysis provides a long-term perspective on diet. It has important advantages in comparison to the stable isotope studies, since it is non-destructive, less expensive and more precise with regard to identifying specific plants.

Until now, researchers detached thick dental deposits from archaeological human teeth for analysis (Fox et al., 1996; Reinhard et al., 2001a,b). Dental calculus consists of microfossils, including a variety of phytoliths (silica or calcium oxalate bodies) and starch grains, trapped into the carbonate calculus matrix (Fox et al., 1996). How exactly the dental calculus

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Fig. 1. Typical thick layer of dental calculus, as seen in burial 12C from Jabuticabeira II.

develops is not yet fully understood (Hazen, 1995). It can be classified as lamellated or homogeneous (Anneroth et al., 1978), depending on the degree of mineralization (Kani et al., 1982).

The microfossils found in dental calculus are crucial to evaluate the use of plants by past populations, especially if they originate from archaeological sites with poor or no plant preservation, such as those found among shellmound (Sambaqui) dwellers from Brazil.

In certain archaeological contexts only a faint cryptic deposit from an originally thick dental calculus preserves. This might be due to taphonomic attrition of the teeth in contact with abrasive sediment such as shells, which destroy the outer layer of the dental calculus. In such cases the remaining dental calculus is only a very thin deposit, impossible to be mechanically detached from the teeth (Fig. 2).



Fig. 2. An incisor from Burial 12C from Jabuticabeira II site, with a thin layer of dental calculus. This type of dental calculus mark was used as sample for the development of the dental wash method.

To avoid the loss of important dietary data in archaeological contexts where calculus preservation is low, we propose a method called “dental wash”. This method is based on the pollen wash technique developed for artifacts (Pearsall, 2003a,b), but instead, uses human teeth with faint marks of dental calculus. A similar procedure is used to remove residual material from the surface of teeth before scanning electron microscope analysis (Ciochon et al., 1990). However, in this case, not the residues, but instead, the cleaned tooth is analyzed. Here we analyzed the residues that were left in the acid solution after the wash, using a similar method to that described by Middleton and Rovner (1994). However, we applied the method to human teeth, not to animal teeth like these researches did. Another difference is the acid concentration. While Middleton and Rovner (1994) used a 10% concentration of hydrochloric acid to warrant dissolving thick dental calculus deposits of herbivore teeth, aiming the extraction of phytoliths, we used only a 4% acid concentration, as we wanted to wash thin dental calculus deposits off human teeth and were concerned about avoiding dental damage. Finally, and most importantly, the goal of this analysis was to determine how many microfossils are trapped in dental calculus. The quantification of microfossils in dental calculus has not been reported in former articles.

For the test of this method, we used teeth from Brazilian shellmound (or Sambaqui) burials. Sambaquis are artificial mounds constructed of faunal remains (mainly mollusk shells), artifacts, habitation structures and burials (Gaspar, 1998). Zooarchaeological and stable isotope studies showed that fishing was the basis of their subsistence (Bandeira, 1992; De Masi, 1999; Figuti, 1993; Klökler, 2001) and that shells were largely construction material. Despite that, recent studies report that plants also played an important role in their diet (Reinhard et al., 2001a,b; Scheel-Ybert, 2000, 2001; Scheel-Ybert et al., 2003).

We here report the experimental results regarding this promising method of recovering microfossils trapped in cryptic dental calculus. We suggest that the future application of this method will recover microfossils that resulted from human dietary and economic activities (including fiber preparation for baskets, mats and ropes) in archaeological contexts formerly excluded from microfossil analyses, due to insufficient quantities of dental calculus.

2. Materials and methods

We selected teeth from two burials (3B and 12C) excavated at the Brazilian coastal Sambaqui Jabuticabeira II, constructed between 2800 to 1805 BP (DeBlasis et al., 1998; Okumura and Eggers, 2005). The teeth from Burial 12C were associated with a young female adult with well preserved, but severely worn, teeth found in an individual burial pit at locus 1.05. The skeleton showed post deposition alterations by roots and rodents. The individual at Burial 3B, from locus 6 B3, was an adult woman with a taphonomically deformed cranium and poor dental preservation, including damaged enamel, broken roots, and faint dental calculus marks. We included burial 3B in our protocol because

we felt that if the method was non-destructive for fragile remains, it would be appropriate for all types of remains.

to estimate the concentration of each type of microfossils in the samples:

$$\frac{(\text{microfossil counting}/\text{Lycopodium counting}) \times \text{number of Lycopodium spores per tablet}^1}{\text{volume}}$$

From burial 12C, four teeth were chosen: a 2nd superior left molar, a 2nd superior right premolar, a 2nd left premolar and a 2nd superior right incisor. Five teeth from burial 3B were selected. They were all molars but because of the bad preservation of the teeth, and the poor preservation of the mandible and the maxilla, it was not possible to identify their anatomical origin.

The teeth were cleaned of loose debris. The teeth of each individual were placed inside separate 50-milliliter beakers with distilled water for 3–5 min to allow the water to penetrate the teeth. The water was decanted and 10–20 ml of 4% hydrochloric acid was added to each of the beakers. The teeth were gently swirled in the acid for 5 min. Then, the teeth from each individual were brushed with a new toothbrush to ensure that the majority of microfossils were loosened. The residues from the brushes were rinsed back into the beakers. The teeth were taken out of the beaker and the acid, washed with distilled water, dried and curated. The residues and the diluted acid in the beakers were transferred to labeled 15 ml centrifuge tubes. The beakers were rinsed into centrifuge tubes. The volume of microscopic remains in the tubes was recorded. Three *Lycopodium* tablets (batch number: 124961) were dissolved in each tube to allow the quantification of microfossils. It is important to note that for the traditional method of processing calculus fragments mechanically detached from teeth, just one tablet of *Lycopodium* is used. Instead of one we used three tablets, since our solution volume is higher due to the washing of various teeth at once and the processing of the obtained solution in one centrifuge tube, not in a microtube. The tubes were centrifuged for 2 min at 1500 RPM. Finally, the hydrochloric acid was decanted, the tube filled with distilled water, agitated with a glass-stirring rod and centrifuged again for the same time at the same velocity as before. The water was decanted and the sediments were rinsed out of the tubes with 95% ethanol and transferred to a labeled vial with a lid.

Five slides were prepared for each of the samples using a drop of glycerin (to permit some movement of the microfossils on the slide) and 10 μl of the sample, so that the total volume analyzed of each sample was 50 μl . The slides were sealed with nail-polish and examined under a light microscope with 20 \times and 40 \times magnification. While scanning systematically the entire slide, type and number of microfossils and number of *Lycopodium* spores were counted. The types of microfossils we expected were based on Reinhard et al. (2001b). They included phytoliths, starch grains, plant fibers, and associations of these fragments. We used a pollen concentration formula (as reviewed by Reinhard et al., 2005)

2.1. Control sample

In order to check if the microfossils found in the experimental teeth samples were not a result of laboratory and field contamination, we made a control sample using tooth brushes formerly employed for cleaning human remains from this shellmound. We applied the same protocol as described above to these tooth-brushes. We anticipated that if microfossils from the teeth came from Sambaqui sediments, or contaminants in the laboratory, we would find these microfossils in the brush preparations.

Again, five slides were prepared with the control sample following exactly the same procedures as described for the experimental samples. They were analyzed using the same light microscope with the aim to count the number of microfossils and *Lycopodium* spores.

3. Results

We found three types of microfossils in both the experimental samples (Fig. 3 and Table 1). Most commonly found were starch grains. In burial 3B and 12C, the starch grain concentrations obtained were 466.98 grains/ μl and 321.43 grains/ μl respectively. The phytolith concentrations were much lower: 14.15 phytoliths/ μl for burial 3B and 6.69 phytoliths/ μl for burial 12C. One diatom fragment was found in the sample from burial 3B.

For the identification of the microfossils, identification keys, reference collections or ethnobotanical studies (as used in the study of Zarrillo and Kooyman, 2006) of the plants found in the region and time of interest are necessary. We could not yet identify the starch grains and the phytoliths found in the samples because there are no published studies on these plant particles within the studied region. Instead, our research aimed at quantifying and not identifying the microfossils. The correct identification of the starch and phytoliths is the aim of a future project.

As for the moment, however, we report that the majority of the starch grains found in the samples (Fig. 3) were spherical (49.24% in burial 3B and 64.64% in burial 12C), with almost always visible centric hila and well defined extinction crosses. We also found irregular (42.42% in 3B and 34.25% in 12C), oval (6.82% only in 3B) and bell-shaped (1.51% in 3B and 1.10% in 12C) starch grains. The size of the grains varied from 10 μm to 15 μm in diameter at burial 3B and from 7.5 μm to 20 μm in diameter at burial 12C. Regarding phytoliths, in burial 3B we just found faceted structures (Fig. 3h)

¹ In this case there were 12,500 spores per tablet.

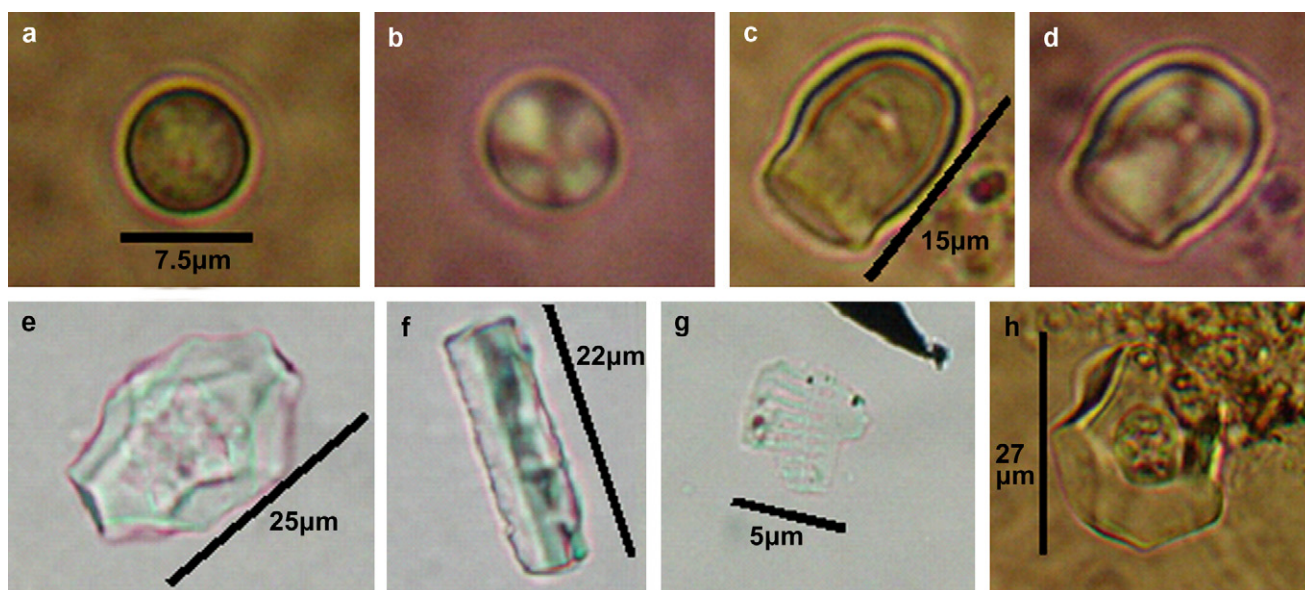


Fig. 3. Microfossils found at 40 \times magnification in the samples from Jabuticabeira II: a) round starch grain (burial 12C); b) the same starch grain viewed under polarized light; c) bell-shaped starch grain (burial 3B); d) the same bell-shaped starch grain viewed under polarized light; e) and f) phytoliths (burial 12C); g) diatom fragment (burial 3B); and h) phytolith (burial 3B).

and at burial 12C we found a druse (Fig. 3e) and two rod shaped phytoliths (Fig. 3f).

No microfossils at all were found in the control samples. This fact indicates that the microfossils found in the experimental sample were most probably not the result of contamination, but instead were microfossils once trapped in the matrix of the thin layer of calculus.

The procedure caused different damage to the teeth, depending on their condition before treatment with HCl. Processing with acid exacerbated the preexisting poor and friable condition of the teeth from burial 3B (Fig. 4a, b). The teeth from burial 12C, however, just became whitened and lost their nacreous appearance (Fig. 5a, b).

4. Discussion

Washing archaeological teeth with cryptic dental calculus with a 4% HCl solution, in fact recovers microfossils once trapped in the calculus matrix. The types of microfossils found were mainly starch grains, but also some phytoliths and a diatom.

In contrast, a former study that employed a similar method reported only the recovery of siliceous plant remains (Middleton and Rovner, 1994). In addition, microfossil studies on lithic instruments (Piperno and Holst, 1998; Piperno et al., 2000;

Barton et al., 1998) reported much lower concentrations of starch than we found in the human teeth. Besides that, the number of phytoliths found in both our teeth samples was lower than that observed in samples from individuals of the late Roman period (Fox et al., 1996), in teeth of an extinct ape (Ciochon et al., 1990) and in herbivore teeth (Middleton and Rovner, 1994). The concentrations of phytoliths in our samples were also very small when compared to that of the starch grains of these same teeth.

These differences might be indicative of a plant diet based mainly on roots and tubers in Jabuticabeira II. In fact, roots and tubers are the major sources of food in many parts of the world (Gott et al., 2006), since, as storage organs of plants, they concentrate reserve starch and thus are very important sources of energy.

In addition, evidences of tubers were found at Jabuticabeira II and other southeastern coastal sambaquis (Scheel-Ybert et al., 2003). All of the tubers found were monocotyledons and some could be identified as *Dioscorea sp* (Scheel-Ybert, 2001). However, many other species must have been used. Furthermore, tuber remains were found in almost all archaeological levels of these sites, and so suggest that they were largely employed by these Sambaqui people. Another line of evidence, such as the lithic industry, shows that in Jabuticabeira II about 10% of the stone artifacts are mortars used for plant processing (Scheel-Ybert et al., 2003), a percentage similar to that found in various other coastal sambaquis (Beck, 1972; Garcia, 1972; Gaspar, 1991; Kneip, 1994; Uchoa, 1973). Finally, it is important to know that tubers “do not generally contain phytoliths” (Gott et al., 2006). So, if both individuals analyzed here ate more roots and tubers than other parts of plants rich in phytoliths (such as leaves or stems), they would have a much higher concentration of starch grains than phytoliths in their dental calculus, as in fact was observed in our samples.

Table 1
Number and concentration of microfossils in each sample

Microfossil	Burial 3B	Burial 12C	Control
Starch grains	99	144	0
Starch grain concentration	466.98 grains/ μ l	321.43 grains/ μ l	0 grains/ μ l
Phytoliths	3	3	0
Phytolith concentration	14.15 phytoliths/ μ l	6.69 phytoliths/ μ l	0 grains/ μ l
Diatom fragment	1	0	0

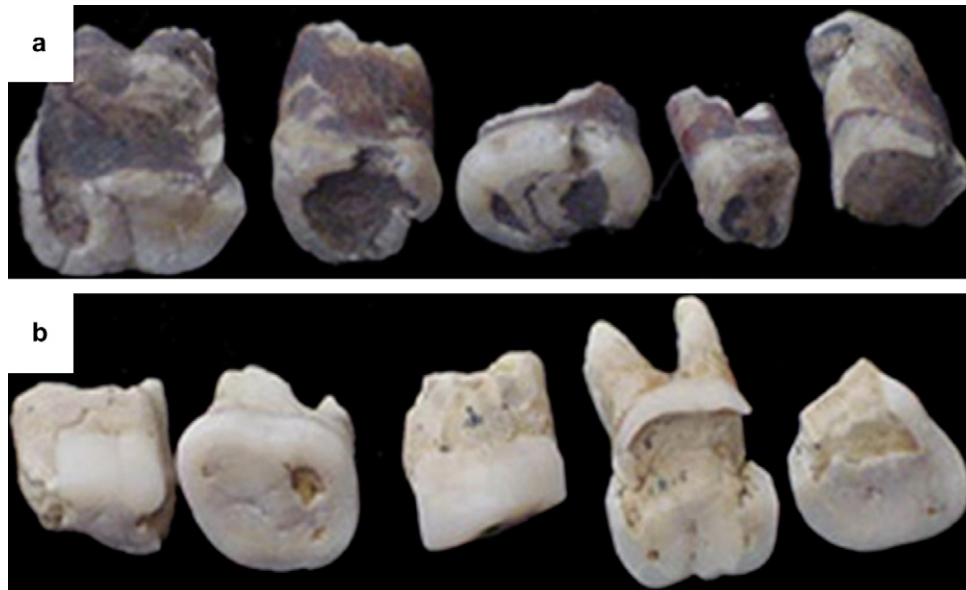


Fig. 4. Teeth from Jabuticabeira II burial 3B: a) teeth that were not used for dental wash, but show the same bad preservation; and b) teeth used for the dental wash (after the procedure was finished).

The difference in starch grain concentration found between the two studied samples (burial 3B: 466.98 grains/ μ l and burial 12C: 321.43 grains/ μ l) could be a result of differences in the diet of the studied individuals. However, this is not the only explanation. This difference could also have arisen due to different teeth chosen for each individual, since we used 5 molars from burial 3B and 1 molar, 2 pre molars and an incisor from burial 12C. Differently from the anterior teeth, the posterior teeth are used for processing the food in the mouth (Pough et al., 2003). Thus, the dental calculus of molars might retain more vegetal microfossils than the anterior

teeth, explaining why the teeth from burial 3B contained more microfossils than those from burial 12C.

Although this analysis of a small sample of teeth provides positive evidence concerning diet, it also provides a cautionary example of potential loss of dental data. In our opinion, any loss of integrity of human remains due to analysis is unacceptable. The wash of the teeth resulted in the recovery of microfossils. On the other hand, we are very concerned about the effects of this process on the surface morphology of human teeth that might prevent other types of analyses, such as microwear studies.

A concentration of 3% HCl in *Gigantopithecus blacki* teeth did not prevent microwear analyses, in the contrary, the acid was used to clean the teeth prior to this kind of analysis (Ciochon et al., 1990). However, a specific study showed that after 30 min exposure of a neolithic human tooth to a solution of 2.5% hydrochloric acid, no alteration was noticed but, after 2 h exposure the enamel was affected and “almost all the microwear features were removed” (King et al., 1999). In the dental wash, the teeth were exposed for just a few minutes, but the solution’s concentration was a little bit higher than that used by King et al. (1999). Thus higher concentration may cause damage to tooth enamel. Microscopic studies on the human teeth washed with HCl will be carried out in the near future to test if microwear studies are still possible after dental wash.

The sediment conditions of Sambaquis are bad for preservation of human and especially plant remains (Scheel-Ybert, 2000, 2001). Thus, the discovery of plant microfossils on teeth in Sambaquis presented here is exciting. However, we must accept that the poor preservation in Sambaquis endangers the integrity of human remains during laboratory work. We think that the basic sediment conditions, combined with local acidity of root penetration results in the degradation of enamel. The application of even dilute acids for limited periods of time results in unacceptable damage to the teeth. Only the main structure of the teeth was maintained,

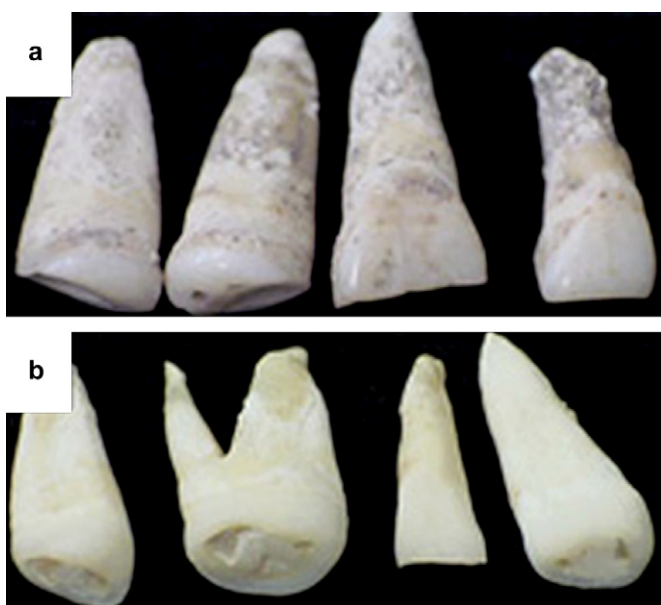


Fig. 5. Teeth from Jabuticabeira II burial 12C: a) teeth that were not used for dental wash; and b) The ones used for the dental wash (after the procedure was finished).

allowing for subsequent paleopathological, metric and non-metric dental analyses.

In conclusion, this study shows that microfossils are in fact trapped in cryptic dental calculus deposits on teeth. These microfossils can be liberated from the surface of the teeth by chemical means. However, the chemical processing used in this study was not gentle enough to be totally non-destructive. We suggest that the solution to this dilemma must be sought in alternative chemical methods or physical separation with sonication.

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